Glutaraldehyde Cross-Linking of the Sheath-Core Structures in Collagen Fibrils of Skin

It is difficult to prepare uniform products such as surgical implants from glutaraldehyde-cross-linked collagenous tissue because gradients of cross-link densities and masses of polyglutaraldehyde form in the usual concentrations of reagent (1% to 6%).[1] Another complication may be differential reactions of the sheath-core structure in the individual fibrils that we have described earlier.[2] To study the reaction of glutaraldehyde with these two regions of the fibril, we stirred 1-mm-thick slices of calfskin reticular dermis (that had been frozen once) with dilute reagent from 0.003% to 0.1% at 22 °C, pH 7.2, for 48 hours, quenched with glycine, and examined them by differential scanning calorimetry and by electron microscopy with positive staining with uranyl acetate/lead acetate and with imbedment in melamine or epoxy resin. Amino acids (for hydroxyproline content and lysine/arginine ratios) were determined on samples that had been reduced with $NaBH_4$ and hydrolyzed with 6 M HCl.

The temperature of denaturation increased to a limit at 87 °C with 0.1% glutaraldehyde (FIG. 1), which gave a product with only 47.2% of the lysine amino groups reacted. The shrinkage tension gave an average molecular mass between cross-links[3] of 10.5 kDa, in reasonable agreement with the 7 kDa calculated from the reacted lysine residues. Low concentrations of glutaraldehyde (0.01%) did not change the general shape or position of the endotherms, but did broaden the high-temperature shoulder. Concentrations near 0.03% reacted with 13% of the lysine amino groups and shifted the sharp (middle) endothermic peak to higher temperatures by 14 °C. Again a broad band of denaturation, extending to 130 °C, appeared. The general features of the endotherms of the treated materials therefore seemed similar to those of the natural materials. The partially denatured treated materials had a different appearance in the electron microscope, however: fibrils seemed to explode progressively along their lengths while remaining discrete. At 83 °C some of the fibrils seemed to have sheaths persisting as films unwrapping from the melted cores, but at no temperature was there the dark-staining material found in untreated tissue,[2] either as cores or as interfibrillar material.

With 0.1% glutaraldehyde (47% reacted lysine) the sharp endotherm is associated with modest (ca. 50% diameter change) swelling of fibrils, again progressively along the length (FIG. 2). The crossbands are retained through the first endotherm, showing

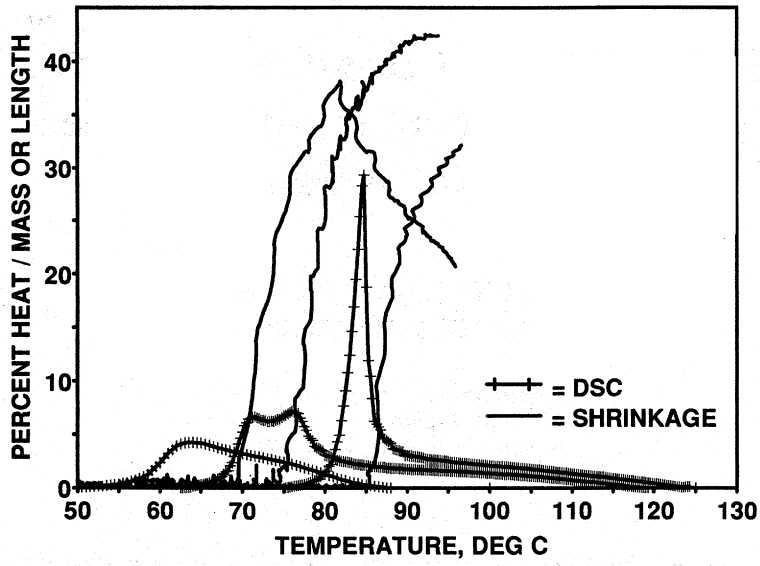

FIGURE 1. Effect of glutaraldehyde cross-linking on differential scanning calorimetric endotherms and percent thermal shrinkage. The levels of glutaraldehyde are, right to left in each family of curves, 0%, 0.03%, and 0.10%.

retention of lateral registration, even after 61% of the collagen has been denatured. As the fibrils swell, the band spacing shrinks. On the second endotherm, lateral structure is gradually lost.

We believe that the sharp endotherm moving from 63 °C in natural fibrils to a maximum of 87 °C in treated tissue is due to melting of molecules that have reacted only intramolecularly. The chemical environments of such cross-links would be as uniform as the structures of the molecules themselves, giving uniform products. Once the cross-linkable lysine sites have been saturated, the stability is maximized, and the corresponding transition temperature stops increasing. The intermolecular packing is much less uniform because of rotational freedom of the molecules with respect to each other. Intermolecularly cross-linked products with a wide range of structures would be formed, with a wide range of stabilities.

REFERENCES

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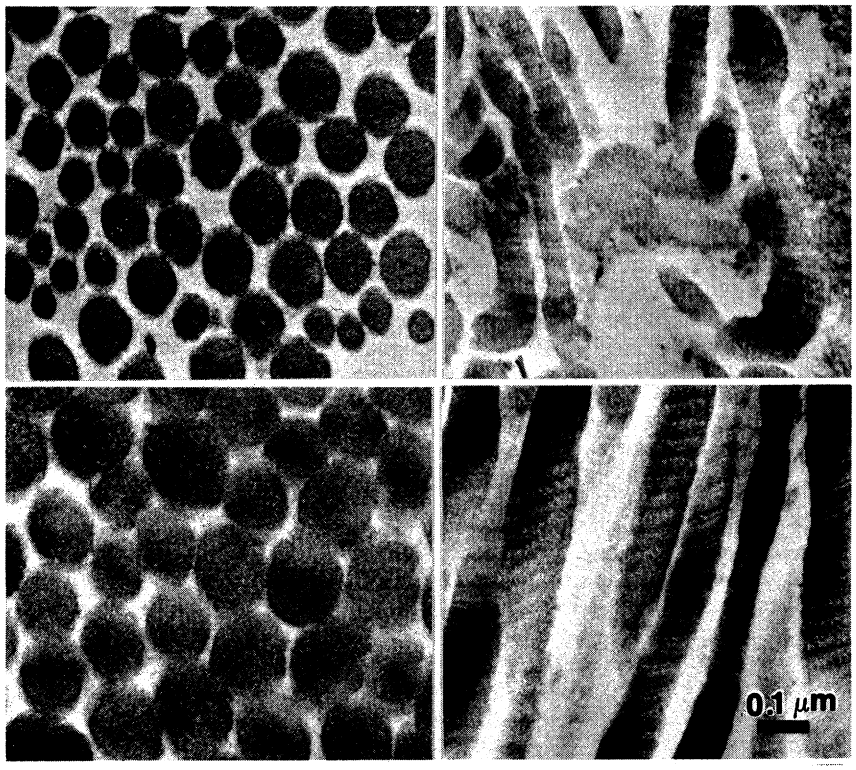

FIGURE 2. Electron micrographs of samples removed from 0.1% glutaraldehyde-treated material at (*upper panels*) 87 °C (endotherm peak) and (*lower panels*) at 90 °C (just above the peak) during the type of temperature scan described in FIGURE 1.